SONY SP6800 (Cell Analyzer)

(SONY, HP)

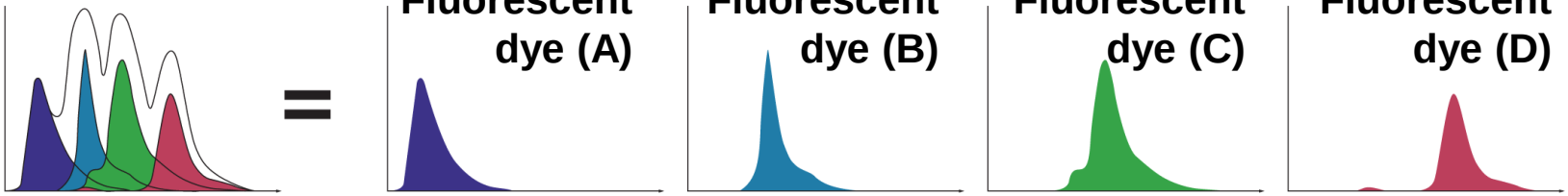
Spectral Analysis Technology

Lasers: Violet (405 nm), Blue (488 nm), Red (638 nm)

Spectral flow cytometer

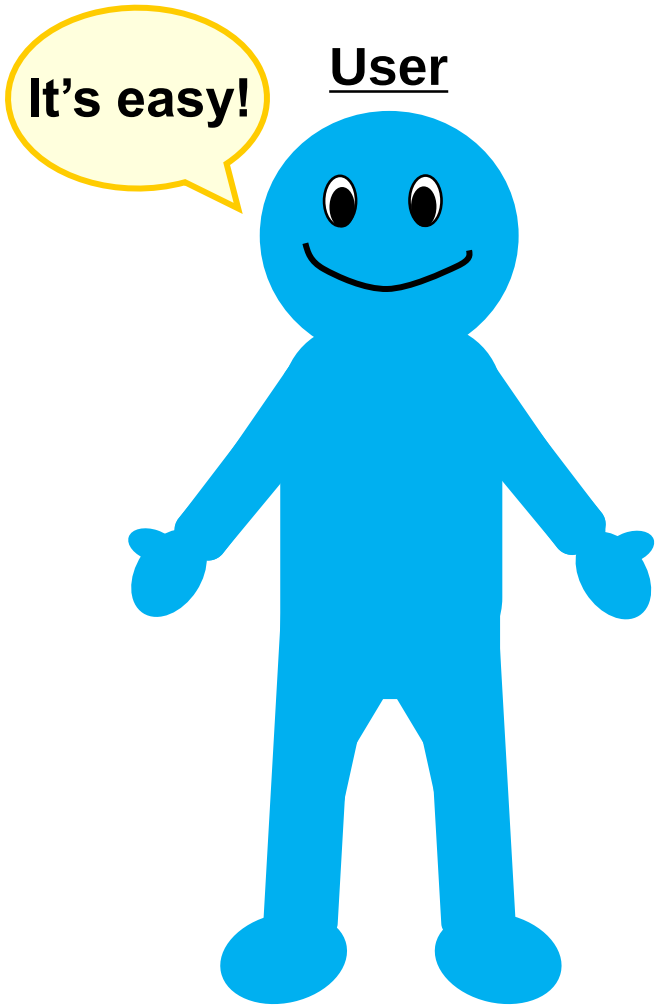
1. Sum the fluorescence together
2. Use unmixing to mathematically separate the colors

Fluorescent dye (A+B+C+D)



1 cell data = 1 line

SONY SP6800 (Cell Analyzer)



1. Turn on the power to start the SP6800
   (PC and SP6800 software)

2. Run “priming” and “Daily QC”

3. Create “Experiment”
   (You can select the colors you use)

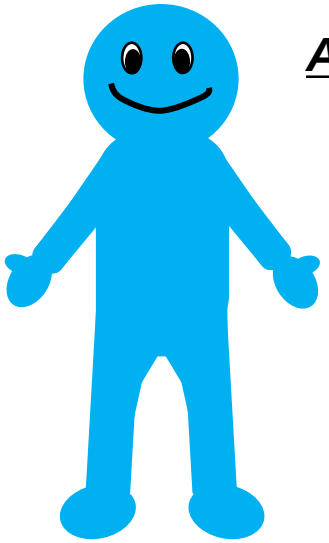
＜First time＞
You need to register the spectral references.

4. Acquire measurement data and analyze data

5. Run “Daily cleaning”

6. Shut down
   (SP software and PC)

SONY SP6800 (Cell Analyzer)



Advantage

No need for a single stain sample
→ You can analyze small samples in multiple colors.

The wavelengths are close…
→ If the waveforms are different, staining is possible.

【Example】

- **We collect the blood sample from mouse. (60 µl)**
- **We prepare these samples.**
  Negative control (30 µl) + Staining sample (30 µl)

CD19-BV421 CD45.2-PE Gr-1-PerCP PI (dead cell)
CD11b-FITC CD45.1-APC CD4/CD8-AF700

SONY SP6800 (Cell Analyzer)

Ogawa lab's spectral reference list

BV421, EGFP, AF488, FITC

PE, PI, PerCP, tdTomato

APC, AF700, BV785

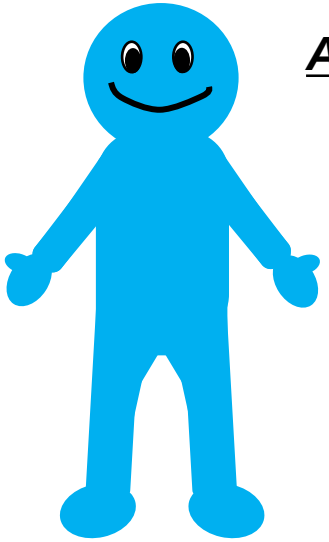
If you want to use these color references, feel free to contact us !!

⬇

User

load spectral reference → no need for single stain ^^

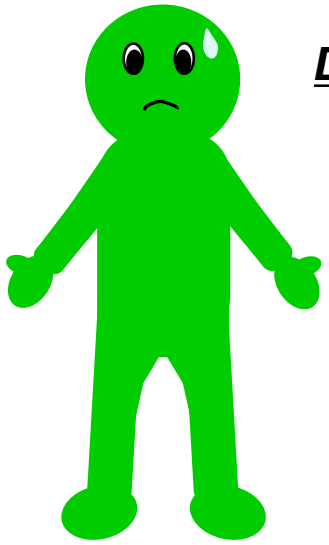
SONY SP6800 (Cell Analyzer)



Advantage

No need for a single stain sample
→ You can analyze small samples in multiple colors.

The wavelengths are close…
→ If the waveforms are different, staining is possible.



Disadvantage

Spectral flow cytometer sums the fluorescence together…
→ You cannot change the voltage of each color.

SONY SH800 (Cell Sorter)

(SONY, HP)

Laser = 4

Violet (405 nm), Blue (488 nm), Yellow-Green (561 nm), Red (638 nm)

Detector = 6 (Choose the filter set you need)

450/50, 525/50, 585/30, 600/60, 617/30, 665/30, 720/60, 785/60

【Example】	Filter Set 1	Filter Set 2
FL1	BV421	BV421
FL2	EGFP	FITC
FL3	tdTomato	PE
FL4	7-AAD	PI
FL5	AF700	APC
FL6	APC-Cy7	AF700

SONY SH800 (Cell Sorter)

1. Turn on the power to start the SH800
   (PC and SH800 software)

2. Follow the "Starting Wizard"
   (chip loading, laser & filter setting)

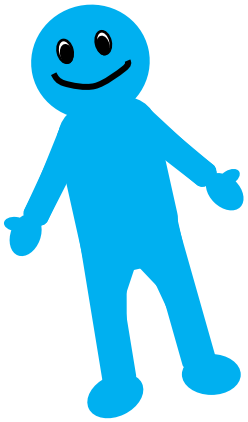
3. Run "Auto Calibration"
   (chip alignment, sort calibration)

4. Create "Experiment"
   (You can select the colors you use)

5. Acquire measurement data and analyze data

6. Follow the "Shut down Wizard"
   (software and hardware; including cleaning)

SONY SH800 (Cell Sorter)



Advantage & Disadvantage

Sorting Chip can be used for 24h
→ Low risk of chip clogging.
→ High cost !! (¥3,000 / chip)

Plate sorting
→ Ex) 17 cells were observed
(20 cells were sorted into 24 well plate)

2-way sorting
※ 4-way sorting → BD

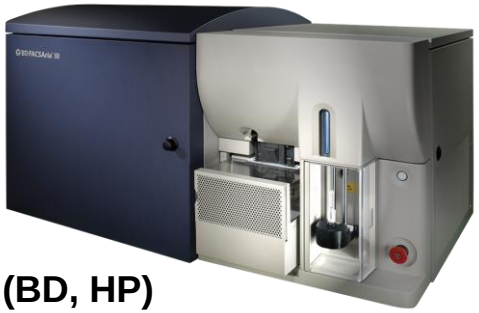
6 detectors
※ 7 colors or more → BD

Longer wavelengths：detection sensitivity is low !!
→ Ex) APC, AF700, etc…：highly expressed antigen

BD FACS in IMEG

BD FACS Aria III

- **3 lasers; Violet (405), Blue (488) and Red (633)**
- **9 detectors**



(BD, HP)

BD FACS Aria SORP

- **4 (+1) lasers; Violet (405), Blue (488), Yellow-Green (561) and Red (633) (+ UV (355))**
- **11 detectors**

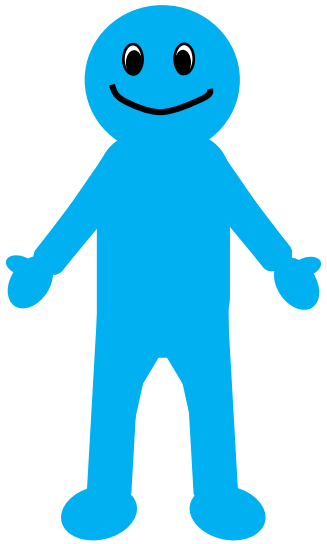
BD FACS Aria IIu

- **3 lasers; Violet (405), Blue (488) and Red (633)**
- **9 detectors**

The advantages of BD FACS

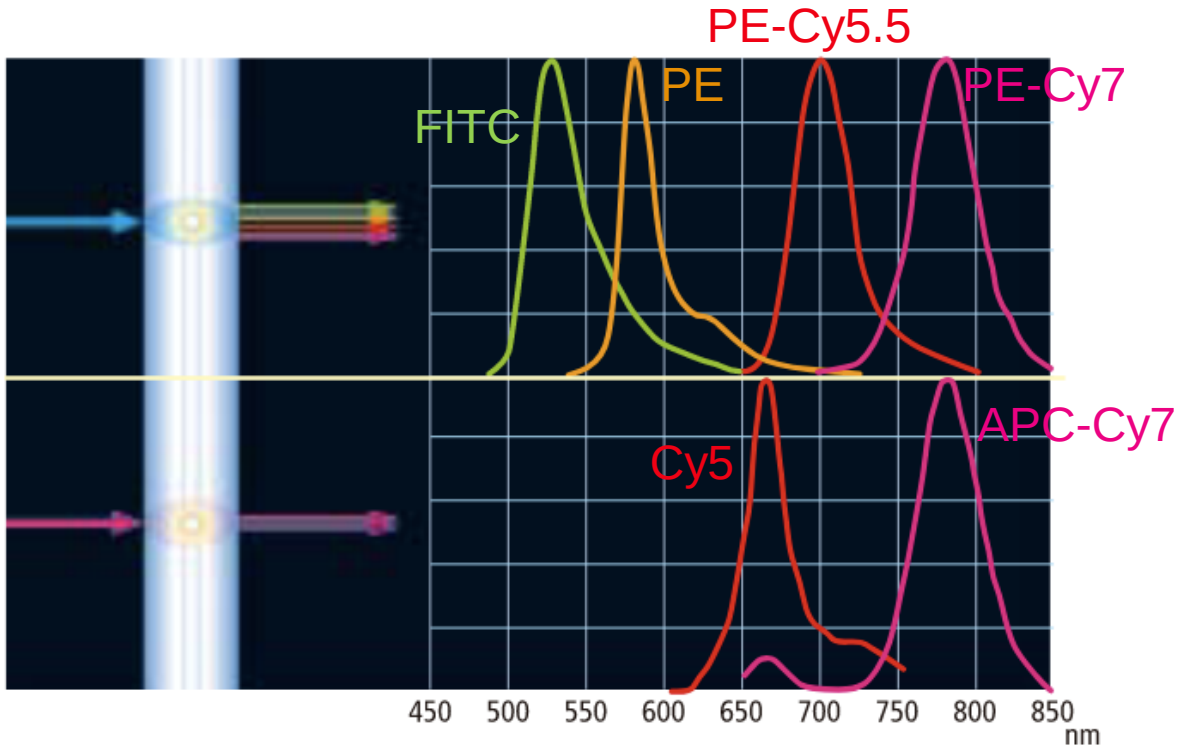
Advantage

- **Spatially-separated spot**
- **4-way sorting**
- **The temperature controlling system**

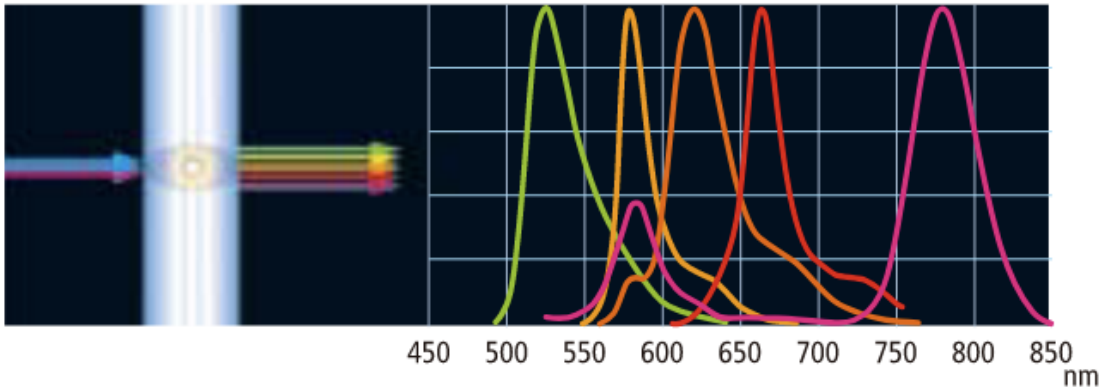


Spatially-separated beam spots

Separated (BD FACS)

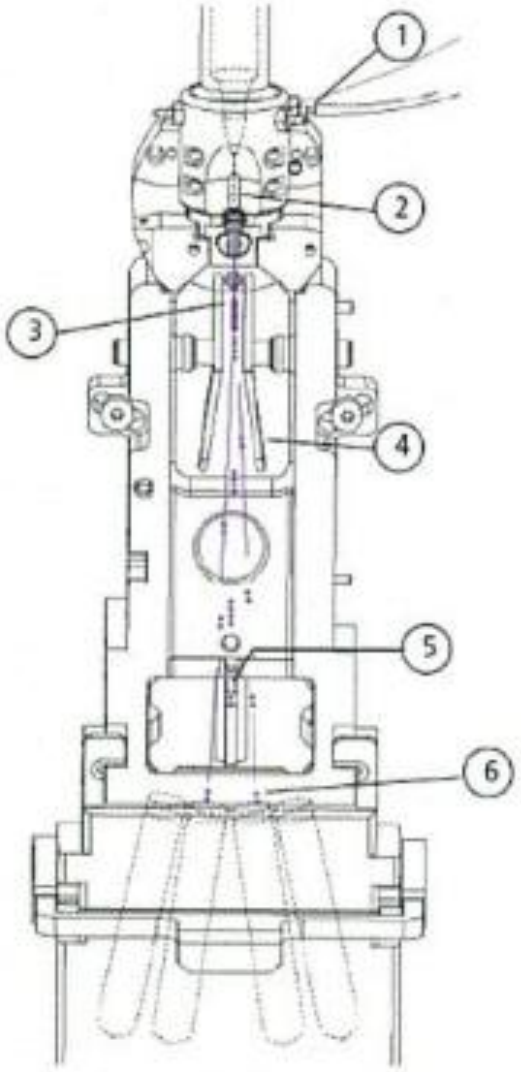


Single (SH800)



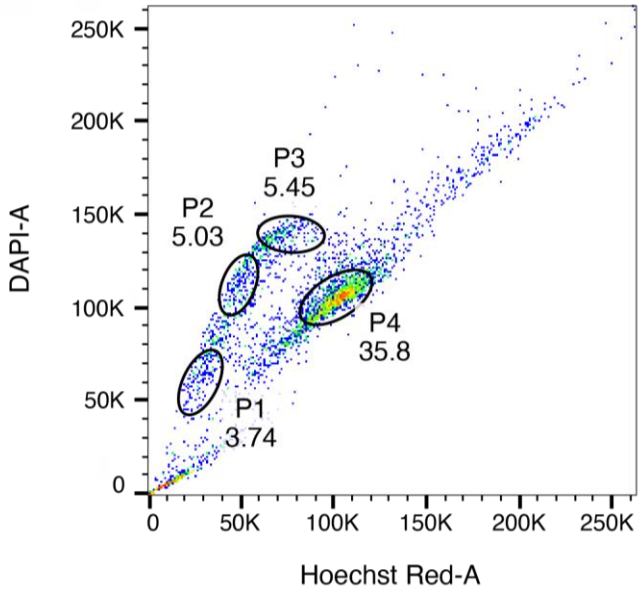
https://www.bdj.co.jp/pdf/64-136-00.pdf

4-way sorting

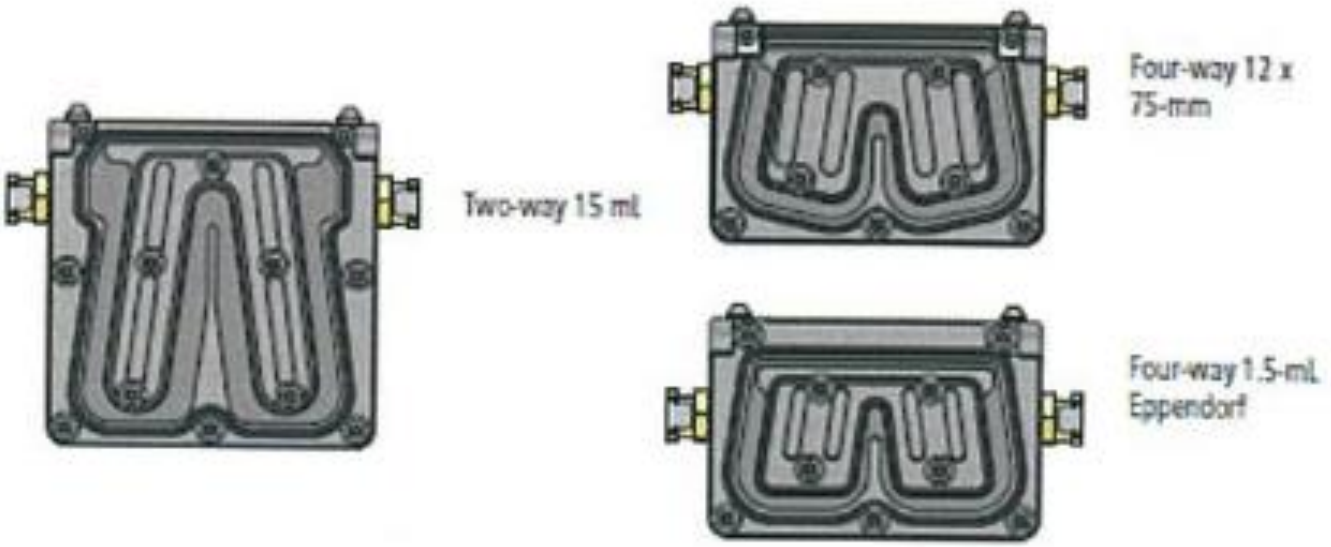


1. Charge is applied via the stream-charging wire in the barb.
2. Sample generates light scatter and fluorescence signal. Signal is analyzed.
3. The charged droplet breaks off.
4. Deflection plates attract or repel the charged droplet.
5. Uncharged droplets pass to waste.
6. Charged drops containing particles of interest are collected.

【Example】



The temperature controlling system



You can keep cells cells at any temperature !!

The disadvantage of BD FACS

Disadvantage

- **Manual setting is required**
  - ✓**Cleaning of Flow Cell**
  - ✓**Nozzle insertion**
  - ✓**stream checking**

  etc....